CHAPTER-I

1. INTRODUCTION

Every developing country in the world, today, is witnessing the phenomena of rural-to-urban migration mainly due to the well accepted notion that opportunities are aplenty in the cities to carve a better life. Kathmandu valley, too, is heading towards rapid urbanization and hence, acutely displays the migration syndrome. This migration syndrome demands to feed a swelling population is one amongst the many. Moreover, with ample international exposure, the eating habits and tastes of people have also come to vary which has further influenced the emergence of numerous restaurants catering to global tastes and cuisines. Thus, there has been a proliferation of varieties of restaurants throughout the cities of developing countries.

Economic factors and employment patterns have led to an increase in consumption of meals outside the home. However, some people prefer to eat outside while others are compelled by circumstances to do so. Hence, there is an urgent need to know the quality of food that is provided in the food establishments.

Microorganisms enter the food by raw ingredients, water, environmental cross contamination, inadequate sanitation and poor handling practices during cooking and serving. Certain microbial contamination of food is an indicator of poor sanitary practice in the preparation and storage of foods. Mishandling in food service establishments can contribute significant outbreak of food-borne diseases (Frazier and Westhoff, 2001).

One of the crucial factor to microbial contamination is the serving of the foods cooked in advance and stored for a long period of time in ambient temperature(FAO, 1992). While serving, they are often not re-heated to a desired temperature to kill the harmful bacteria which can ultimately lead to high total plate count as well as elevated coliform and faecal coliform counts.

The control of microorganisms is needed to retard or prevent spoilage and to reduce or eliminate health hazards associated with foods. Although the control of microorganisms in food is usually relegated to the food processor, everyone involved in the production, processing, handling, retailing, preparation, and serving should be involved in the control process as well as in maintaining a safe and nutritious food supply. Moreover, keeping the contamination low by sanitary means is very important.

The need for this overall effort is evident from the fact that only few outbreaks of foodborne illness are caused by problems at the processing level. Most of the outbreaks are caused by mishandling and contamination of foods at foodservice establishments or in the home (Banwart, 1998).

The food can be guaranteed of microbial safety only when overall processing, including the production of raw materials, distribution and handling by the consumers are taken into considerations. Therefore, the microbiological quality assurance of food is not only a matter of control but also a careful design of total processing chain.

Microbial examination of final product does not reveal information of the point of contamination nor ensures protection against it but only gives the idea of hazard quality. For this reason, the traditional approach of the hygiene supervision is not quite effective and is replaced by a more programmatic approach focused on the control of factors threatening the wholesomeness already during the production process (Jay, 1992).

A relative new concept has developed known as Hazard Analysis Critical Control Point (HACCP) which is a scientific and systematic approach of identification, assessment and control of hazardous pathogens. The system seeks to identify the hazards associated with any stages of food production, processing or preparation, assess the related risks and determine the operation where control procedures will be effective. Emphasis is placed on assessing hazards, risk and identifying critical control points rather than on control criteria and monitoring.

The HACCP concept was originally proposed for the food processing industry. However, available surveillance data suggest that the incidence of food borne disease outbreaks caused by mishandling of foods is actually higher in food service establishments and at the consumer level than in the food processing industry. Therefore, the HACCP concept has been extended to food service establishments (Bryan, 1981) and even to the home (Zottola and Wolf, 1980).

The system offers a rational approach to the control of microbiological hazards in foods avoids many weaknesses inherent in the inspectional approach and circumvents the shortcoming of the reliance on microbiological testing. HACCP system places emphasis on the quality of all ingredients and process steps on the premise that safe product will result if these are properly controlled. The system is thus designed to control organisms at the point of production and preparations. Thus, the central feature of HACCP is the determination of the CCPs which is an operation (practice, procedure, location or process) at which control can be exercised over one or more factors to eliminate prevent or minimize a hazard to ensure the safety of products.

Moreover; HACCP can be applied throughout the food chain from the primary production to processing, manufacturing, distribution and retail to the final point of consumption. The benefits include the enhanced food safety and more timely response to problems (FDA, 2005).

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To assess the hazard analysis critical control point (HACCP) of fast food (*momo*) from different restaurants of Kathmandu Metropolitan City.

2.2 SPECIFIC OBJECTIVES

1. To study the preparation process of chicken *momo* and buff *momo* and their hygienic status.
2. To obtain information about hazards associated with preparation and storage of these food items.
3. To determine the CCPs of the food items.
4. To find out the control measures for these CCPs.
5. To assess the environmental condition of those restaurants.
6. To compare the microbial safety of food between different restaurants.

CHAPTER-III

3. LITERATURE REVIEW

3.1 BACKGROUND

3.1.1 Food safety hazard

Hazards are biological, physical, or chemical agents that may cause food to be unsafe for human consumption. Because many foods are agricultural products and have started their journey to our door as animals and plants raised in the environment, they may contain microscopic organisms. Some of these organisms are pathogens which mean that under the right conditions and in the right numbers, they can make someone who eats them sick (FDA, 2005).

Foods can become contaminated by toxic chemicals or toxins in our establishment or in the environment. Physical objects may also contaminate food and cause injury. Some foods undergo further processing and at times, despite best efforts, become contaminated. These inherent hazards, along with the hazards that may be introduced in our establishment such as metal fragments from grinding can lead to injury, illness, or death.
The Centers for Disease Control and Prevention (CDC) Surveillance Report for 1993-1997, "Surveillance for Foodborne-Disease Outbreaks - United States," identifies the most significant contributing factors to foodborne illness. Five of these broad categories of contributing factors directly relate to food safety concerns within retail and food service establishments and are collectively termed by the FDA as "foodborne illness risk factors." These five broad categories are:

- Food from Unsafe Sources
- Inadequate Cooking
- Improper Holding Temperatures
- Contaminated Equipment
- Poor Personal Hygiene

3.1.2 Hazard analysis critical control point (HACCP)

3.1.2.1 History and background of HACCP system

Hazard Analysis Critical Control Points (HACCP) system originated in the chemical processing industry, particularly in Great Britain, over 40 years ago. Then in the 1950s, 1960s and 1970s, the Atomic Energy Commission made extensive use of HACCP principles to design nuclear power plants (Snyder, 1992).

The modern concept of HACCP system for food safety was pioneered in the 1960s by the Pillsbury Company, the United States National Aeronautics and Space Administration (NASA) as a collaborative development for the production of safe foods for the United States Space Program. NASA wanted a “Zero defects” program to guarantee the safety of the foods that astronauts would consume in space (Mayes, 1993).

Pillsbury presented the HACCP concept publicly at a conference for food protection in 1971 and in following year conducted a three week workshop for Food and Drug Administration (FDA) inspection which culminated in the use of HACCP principles in the promulgation of low acid canned foods regulation in 1974. This remains as the only instance in which HACCP principles have been written into a federal regulation for food production (Sperber, 1991).

The development of HACCP program appeared very simple and in the 1970s and in early 1980s, the HACCP approach was adopted by some major food companies and began to receive attention from segments of the food industry other than manufacturing International Commission on Microbiological Specification of Foods (ICMSF), revealed growing international awareness of the HACCP concept and its usefulness in dealing with food safety (Sperber, 1991).

In 1980, National Academy of Science (NAS) was requested to formulate the general principles for the application of microbiological criteria for foods. In its assessment of the effectiveness of food regulation in the United States, NAS subcommittee recommended in 1985 that the HACCP approach be adopted by regulatory agencies. This recommendation led to the formation of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) in 1987. The committee expanded the HACCP protocol from its original three principles to seven principles.

In 1989, USDA’s Food Safety and Inspection Service (FSIS) initiated a two years HACCP implementation study in which it would evaluate the effectiveness of HACCP programs in providing a greater assurance of the safety of meat and poultry products. More recently, The International Association of Milk, food and Environmental Sanitarians (IAMES), has recommended the broad application of HACCP to food safety.

3.1.3 Definition of terms

The hazard analysis critical control point (HACCP) system identifies specific hazards and preventive measures for their control to ensure the food safety. HACCP (pronounced “Hassap”) is a tool to assess hazards and establish control systems that focus on preventive measures rather than relying mainly on end-product testing. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments. (FAO/WHO, 1993).

HACCP: A system which identifies specific hazards and preventive measures for their control.

Hazards: Hazards are the pathogens, toxins, chemical residue or injurious foreign material which may be present in any stage of product’s life and are the potential to cause the harm. So hazards can be biological, chemical or physical.

Risk: Risk is the probability that the hazards will be realized or will happen. The risk may be medium, high or low based on the judgement of experience.

Critical Control Points (CCPs): A CCP is an operation (practice, procedure, locations or process) at which control can be exercised over one or more factors to eliminate, prevent or minimize a hazards to acceptable levels.

Critical limits: A value which separate acceptability from unacceptability.

Corrective actions: The action to be taken when the results of monitoring the CCPs indicate a loss of control.

Monitor: To conduct a planned sequence of observation or measurements to assess whether a CCP is under control.

Hence, now HACCP can be defined as a science based system, which identifies, evaluates and controls hazards, which are significant for food safety. It is world-wide recognized systematic and preventive approach that addresses hazards through anticipation and prevention, rather than relying mainly on end-product inspection and testing. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments.

The HACCP system can be applied throughout the food chain from the primary producer to the final consumer. Besides enhancing food safety, other benefits in applying HACCP include more effective use of resources and more timely response to food safety problems. In addition, the application of the HACCP system can aid inspection by food control regulatory authorities and promote international trade by increasing consumer confidence in food safety.

3.1.4 HACCP principles

The HACCP protocol of 1971 consisted of three principles:

1) Hazard analysis and risk assessment
2) Determination of critical control points (CCPs)
3) Monitoring of CCPs

NACMCF (National Advisory Committee on Microbiological Criteria for Foods (1989) added four principles to the original protocol, producing the following seven principles (Sperber, 1991).

Principle 1

Conduct a hazard analysis.
Identify the potential hazard(s) associated with food production at all stages, from primary production, processing, manufacture and distribution until the point of consumption. Assess the likelihood of occurrence of the hazards (risk assessment) and identify preventive measures for their control (risk management).

Principle 2

Determine the Critical Control Points (CCPs). Determine the points, procedures or operational steps that can be controlled to eliminate the hazard(s) or minimize their likelihood of occurrence; these are the Critical Control Points (CCPs). Two points of CCP are identified.

CCP_1 that will assure control of a hazard.
CCP_2 that will minimize but can not assure the control of a hazard.

A “Step” means any stage in food production and / or manufacture including the receipt and / or production of raw materials, harvesting, transport, formulation, processing, storage, etc. Normally, CCPs may be raw materials and ingredients, moisture, oxidation-reduction potential, hydrogen-ion concentration, food additives, time-temperature and environmental factors.

Principle 3

Establish critical limit(s) which must be met to ensure the CCP is under control.
Once the critical control point have been identified it is important to select appropriate means to check that the hazard has been controlled at the critical control point. Thus, control measures, should be implemented at each critical control points. These measures must be applicable, practical and economically feasible and must ensure the safety of the food for each point; criteria must be specified that will ensure the safety of the product. Monitoring the end point temperature after heat processing; time and temperature exposure adequate to inactivate microorganism of concern; pH of fermented foods; humidity in storage area for dry products; temperature during distribution of chilled foods; depth of product to be chilled; instruction on labels of finished products describing recommended procedures for preparation and use by the consumer.

Principle 4

Establish a system to monitor control of the CCP by scheduled testing or observations.
Monitoring involves the systematic observation, measurement and or recording of the significant factors for control of the hazard. Monitoring must detect any deviation from the specification (loss of control) in time for corrective action to be taken before the product is sold or distributed. Five main types of monitoring are employed - Observation, Sensory evaluation, Measurement of physical and chemical properties, Chemical testing and Microbiological testing.

Principle 5

Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

If monitoring indicates that a process is out of control or that established criteria are not met, immediate corrective action must be taken. The specification depend on the process being monitored and may include reheating or reprocessing, increasing temperature, decreasing water activity, decreasing pH, extending the process time, adjusting the process at a later stage, rejecting incoming lots, discarding the product. The decision will be based on the hazards, their severity and the risks involved and on the expected use of the product.

Principle 6

Establish procedures for verification, which include supplementary tests and procedures to confirm that the HACCP system is working effectively.

Principle 7

Establish documentation concerning all procedures and records appropriate to these principles and their application.

3.1.5 Application of HACCP principles (FAO/WHO, 1993)

During the hazard analysis and subsequent operations in designing and applying HACCP systems, considerations must be given to the impact of raw materials, ingredients, food manufacturing practices, role of manufacturing processes, consumer population at risk and epidemiological evidences relative to food safety. The intent of the HACCP system is to focus control at CCPs. Redesign of the operation should be considered if a hazard is identified but no CCPs are found. The HACCP application should be reviewed and necessary changes made when any modification is made in the product, process, or at any step of manufacturing. The application of HACCP principles requires the following tasks as identified in the logical sequence for application of HACCP.

3.1.5.1 Assemble HACCP team

The food operator should assure that the appropriate product specific knowledge and expertise is available for the development of an effective HACCP plan. Optimally, this may be accompanied by assembling a multidisciplinary team which should include as appropriate expertise in agronomy, veterinary health, microbiology, public health, food technology, environmental health, engineering, etc. according to the particular situation.

3.1.5.2 Describe product

A full description of the product should be drawn up, including relevant safety information such as: composition, physical/chemical structure (including water activity, pH etc.), packaging, durability and storage conditions and method of distribution.

3.1.5.3 Identify intended use

The intended use should be based on the expected uses of the product by the end user or consumer. In specific cases, vulnerable groups of the population e.g. institutional feeding may have to be considered.

3.1.5.4 Construct flow diagram

The flow diagram should be constructed by the HACCP team. The flow diagram should cover all steps in the operation. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specified operation.

3.1.5.5 On-site verification of flow diagram

The HACCP team should confirm the processing operation against the flow diagram during all stages and hours of operation and amend the flow diagram where appropriate.

3.1.5.6 List all potential hazards associated with each step and consider any preventive measures to control hazards (Principle 1)

The HACCP team should list all hazards that may be reasonably expected to occur at each step from primary production, processing, manufacture and distribution until the point of consumption. The HACCP team should next conduct a hazard analysis to identify for the HACCP plan, which hazards are of such a nature that their elimination or reduction to acceptable levels is essential to the production of a safe food. During conduction of hazard analysis, wherever possible the following points should be included:

- The likely occurrence of hazards and severity of their adverse health effects
- The qualitative and / or quantitative evaluation of the presence of hazards
- Survival or multiplication of microorganisms of concern

- Production or persistence in foods of toxins, chemicals or physical agents and
- Conditions leading to the above

The HACCP team must then consider what control measures, if any, exist which can be applied for each hazard. More than one control measure may be required to control a specific hazard(s) and more than one hazard may be controlled by a specified control measure.

3.1.5.7 Determine critical control points (Principle 2)

There may be more than one CCP at which control is applied to address the same hazard. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree (Flow Chart 2) which indicates a logic reasoning approach. Application of decision tree should be flexible, given whether the operation is for production, slaughter, processing, storage, distribution or other. It should be used for guidance when determining CCPs. This example of a decision tree may not be applicable to all situations and other approaches may be used.

If a hazard has been identified at a step where control is necessary for safety, and no control measure exists at that step, or any other, then the product or process should be modified at that step, or at any earlier or later stage, to include a control measure.

3.1.5.8 Establish critical limits for each CCP (Principle 3)

Critical limits must be specified and validated if possible for each critical control point. In some cases, more than one critical limit will be elaborated at a particular step. Criteria often used include measurement of temperature, time, moisture level, pH, water activity, available chlorine and sensory parameters such as visual appearance and texture.

3.1.5.9 Establish a monitoring system for each CCP (Principle 4)

Monitoring is the scheduled measurement or observation of a CCP relative to its critical limits. The monitoring procedures must be able to detect loss of control at the CCP. Further, monitoring should ideally provide this information in time to make adjustments to ensure control of the process to prevent violating the critical limits. Most monitoring procedures for CCP will need to be done rapidly because they relate to on-line processes and there will not be time for lengthy analytical testing. That's why physical and chemical measurements are often preferred to microbiological testing. All records and documents associated with monitoring CCPs must be evaluated by the designated person with knowledge and authority doing the monitoring and by a responsible reviewing to carry out corrective actions when indicated.

3.1.5.10 Establish corrective actions (Principle 5)

Specific corrective actions must be developed for each CCP in the HACCP system in order to deal with deviations when they occur. The actions must ensure that the CCP has been brought under control. Actions taken must also include proper disposition of the affected product. Deviation and product disposition procedures must be documented in the HACCP record keeping.

3.1.5.11 Establish verification procedures (Principle 6)

Establish procedures for verification. Verification and auditing methods, procedures and tests, including random sampling and analysis can be used to determine if the HACCP system is working correctly. The frequency of verification should be sufficient to confirm that the HACCP system is working effectively. Examples of verification activities include:

- Review of the HACCP system and its records
- Review of deviations and product dispositions

- Confirmation that CCP are kept under control

Where possible validation activities should include actions to confirm the efficacy of all elements of the HACCP plan.

3.1.5.12 Establish documentation and record keeping (Principle-7)

Efficient and accurate record keeping is essential to the application of an HACCP system. HACCP procedures should be documented. Documentation and record keeping should be appropriate to the nature and size of the operation.

Documentation examples are:

- Hazard analysis
- CCP determination
- Critical limit determination

Record examples are:

- CCP monitoring activities
- Deviations and associated corrective actions
- Modifications to the HACCP system

3.1.6 Brief history and definition of *MOMO*

Dumplings (*Momos*) are popular in Nepal, Sikkim and Tibet. *Momos* are made of simple flour and water dough, white flour is generally preferred and sometimes a little yeast or baking soda is added to give a more doughy texture to the finished product. The filling may be one of the several mixtures of minced buff/ pork/ chicken/ or vegetables with any or all of the following: onions, shallots, garlic and cilantro/coriander. The mixture is usually spiced with salt, pepper and often ground cumin. This meat mixture is wrapped in a circular sheet of elastic dough into a fashionable ball and is steamed for 15-20 minutes before serving hot with soup and pickle.

3.1.7 Application of HACCP approach

HACCP is an internationally recognized tool for managing the safety aspect of the production, processing, distribution and preparation of food. In 1982, ICMSF and WHO met to discuss HACCP and its applications in food hygiene and concluded that HACCP approach can be applied to food safety in homes as well as in processing and food service establishments.

Giomo *et al*. (1990) conducted a study on microbiological criteria restoration. The study was carried out as a service mean for checking the application of HACCP system. For this purpose, microbiological end-product survey was conducted in food service establishment where the HACCP concept was applied. Microbiological limits suggested were total aerobic count of 10^5cfu/gm, 50cfu/gm of fecal coliform, 10^2cfu/gm of *S. aureus* and 10cfu/gm of sulfite reducing Clostridia.

Scott *et al.* (1990) investigated on the survival and transfer of bacteria from laminated floor spaces and cleaning cloths under laboratory condition. Drying of the laminated surfaces showed substantial reduction in the recoverable organisms, but on the soil surfaces and on clean and soiled clothes, gram-positive and gram-negative species survived for four hours and in some cases up to 24 hours. They suggested that contact of fingers of utensils with these contaminated surfaces may transfer sufficient number of such organisms and eventual contact with food may represent a potential hazard.

Smith *et al.* (1990) applied HACCP approach to sous vide (vacuum processing technology) prepared meat/ pasta product to meet the increasing consumers' demand for microwavable convenience foods with extended shelf life. The CCPs suggested to control the identified microbiological hazards included quality of raw materials, time-temperature relationship, sanitation and packaging control and incorporation of additional barriers like pH, water activity reduction in the formulated product.

Tompkin (1990) applied HACCP system in the production of meat and poultry product and concluded that the risk of food borne illness could be reduced through implementation of the HACCP.

Bryan *et al.* (1992) conducted a hazard analysis of vending operations at a railway and a bus station in a large city of Pakistan. Commonly prepared foods which were studied included rice, pulses, chickpea, ground meat and potato mixture, meat strew and okra. Large number (10^{4-7}) of *Clostridium perfringens* were isolated from samples of ground meat dishes, pulses and chick peas collected during display. Aerobic colony counts were found high in all those items unless hot at temperature>55°c. Cooking was usually found to be thorough, but spores survived which germinated during display. High temperature holding or periodic reheating maintained safe food and were the suggested CCPs for the operation.

Bryan *et al*. (1992) conducted hazard analysis of vending operations of chicken, rice, pulse patties and ice-cream in a resort town in Pakistan. Time –Temperature exposure during cooking was found to be adequate but recontamination from cutting board, knives and hands of the vendors were suggested. Buffalo milk was held in a freezer and not boiled by the vendors. Hence pathogens are not killed; milk shakes are found to be risky. Pulse patties were not always thoroughly cooked, so pathogens could have survived. Holding stacks of them allow germination and growth of bacterial spores. Thus, the report recommended that Health agency personnel, vendors, consumers of these foods must be informed of the hazards and appropriate preventive measures.

Bryan *et al*. (1991) conducted hazard analysis in 13 homes in each of a valley and a town in Pakistan. Pulses, lentils, chick pea, potatoes, rice and combination of them, curd and weaning preparations were commonly prepared in both locations, and meat dishes were prepared in town. From the study, it was found that cooked foods were left at room temperature overnight in 50% of the homes with elevated aerobic count to be 10^{6-9}cfu/gm, 77% of sample showed coliform count more than 10^{5}cfu/gm. Greater than

10^4cfu/gm of *S. aureus* from curd and buffalo milk. *Cl. perfringens* were isolated from 18% of samples, once from pulse left overnight in quantities exceeding 10^7cfu/gm. CCPs found were cooking, manipulation of foods after cooking, holding cooked foods and reheating.

Ferrari (1992) applied HACCP in public catering service to overcome the hazards in public catering service and concluded that the effective HACCP can be established only when health education is given to the employees.

Benezet *et al.* (1993) reported that the quality of “dry curd sausages”, of two meat product company in Spain showed considerable improvement with the introduction of HACCP system.

Tarwate *et al.* (1993) conducted an investigation to analyze microbiological hazard and to determine the CCPs in the buffalo slaughter line, in which nine different sampling points showed significant differences. The maximum levels of contamination amongst slaughterhouse points were noted for floors, platforms and walls. Thus, floors, platform, walls, knives, axe, saw blade and handsaws were considered as CCPs in the slaughter.

Berends *et al*. (1994) applied HACCP approach in meat production and concluded that a future implementation of the approach in the entire chain of meat production i.e. from conception to consumption is possible.

Gerigh and Ellerbrock (1994) applied the HACCP system in food production. In the application of HACCP system, they reported the start as the collection data, a risk assessment, the determination of critical control points, the specification of critical limits to separate acceptable and unacceptable values for each CCP and finally keeping of records and the verification to the implemented HACCP concept.

Notermans *et al.* (1994) presented an approach that permitted the identification of potentially hazardous bacteria based on a list of all these bacteria which are known to cause food borne disease in man. Following the evaluation of raw materials, the production process, possibilities of contamination etc., deletion from or addition to the list were made. Thus, more precise evaluation of hazards would be made during identification of CCPs and the setting of a control criterion at each CCP.

Shanaghly *et al.* (1994) revealed that continuous monitoring showed an improvement in the microbiological quality of the food. They reported that more than 90% of samples showed total viable count of less than 10^3cfu/gm and the virtual elimination of pathogens when HACCP was used. Thus with the introduction of HACCP, there was a reduction in the level of sampling and provided a useful set of records for quality assurance.

Weingold *et al*. (1994) reviewed the food borne disease outbreak in New York State to develop two new categories by which food borne disease vehicles. They classified it as methods of preparation and significant ingredients and data grouped by this method could be used readily for the hazard analysis, identification of CCPs and establishing critical limits. By identifying these features in a system that closely relates to the food preparations practices, corrective action could be taken to reduce or eliminate the occurrence of illness from that particular food. Thus, they concluded that increase support of food borne disease surveillance would provide the data needed to make the system a valuable tool for use in HACCP risk assessment.

Gill (1995) showed HACCP system to assure the hygienic conditions of red meat and concluded that the general hygienic condition of meat would be improved only if effective HACCP system could be developed for meat production, preparation and distribution processes. The development of effective HACCP systems is impeded by the uncertain commitment of management to product improvement, the lack of defining procedures for the objective identification of hazardous practices and the persistence of subjective assessment of the hygienic condition of product.

Limbiri *et al*. (1995) applied HACCP in a flight catering establishment following an outbreak of Salmonellosis affecting 415 passengers on flight in 1991, the associated flight catering establishment located on a Greek island was surveyed for two years. During the first year of the survey the bacteriological quality of food was not satisfactory. In attempt to minimize the food safety for crew and passengers, the HACCP approach was implemented in 1993. Since its application, greatly supported by the management and staff, the bacteriological quality of aircraft meals was considerably improved.

Abdulla *et al.* (1996) conducted HACCP in school food programs in Bahrain. Hazard analysis was conducted in 6 food preparation sites and 16 school canteens in the state of Bahrain. Sandwiches made with cheese, meat, eggs, liver and beef are prepared in small shops outside schools. Foods were cooked between 4 and 5 a.m. Time-temperature exposure during cooking was adequate to kill vegetative cells of microbes and their spores, but potential for recontamination existed from the hands of food workers, utensils and clothes.

Joshi (1996) applied HACCP to traditional Nepalese meat based street foods- "Kachila", "Chhoyala", "Wo of green gram with meat" and "Sekuwa". The samples of raw materials, various stages of processing steps and final stage of these products were microbiologically investigated to determine the Critical Control Points (CCPs) and control measures were implemented properly to maintain the hygienic condition of these foods.

Ram *et al.* (1996) carried out the study on Microbiological quality and incidence of organisms of public health importance in food and water in Ludhiana, India. Bacteriological analysis of 713 samples of various types of food and potable water samples were carried out. The highest count ranging from 2.5×10^6-7.5×10^6 organisms/gm were observed in raw vegetables and fruits, followed by 3×10^6-$9.8 \times$

10^7/ml, 8.3 × 10^4-8.9 × 10^7/ml and 1 × 10^3-6.7 × 10^7/g in fruit juices ,milk and its products and salty non milky snacks respectively. Fresh Chapati, Dal, Rice, Cooked vegetables and Curry etc. showed no microbial contamination. However, samples from road side cafes gave counts up to 1 × 10^7 org/gm. Among 1332 isolates, 16 types of organisms of public health significance were obtained. They were enterotoxigenic *E. coli* (55), *E. coli* 0157 (3), enteropathogenic *E. coli* (1), enterotoxigenic *Klebsiella* (23), *Streptococcus fecalis* (152), *Bacillus cereus* (133), *S. aureus* (125), *Aeromonas* spp. (57), *Salmonella* spp. (10), *Shigella* spp. (4) and *Yersinia enterocolitica* (2).

Sierra *et al.* (1996) applied HACCP program in the sheep processing line that enhances the microbiological safety of the product. Microbiological analysis of viable counts and enterobacteriaceae to assess the slaughtering hygiene along the processing line were done. The stage of evisceration can be regarded as a critical point within the HACCP system for lamb slaughtering operations.

Victor *et al.* (1996) conducted HACCP to raw pork and chorizo (fermented pork sausage) on retail sale in variety of outlets in Mexico City. Total bacterial counts and enterobacteriaceae counts were determined and the sample were analyzed for the presence of *Salmonella* spp. Pork sold from refrigerated display cabinets in supermarket and butcher shop was poor microbial quality similar to that sold in street markets. In all types of outlets, a high proportion (76%) of samples contained *Salmonella* spp. It is apparent that both animal husbandry and slaughter procedures for pig require further study as does the pork-processing industry in order to define how the next become so heavily contaminated.

Garcia *et al*. (1997) applied HACCP to catering in Spain. This approach is useful for correcting failures and factors that contribute to foodborne diseases as well as to implement the appropriate preventive action.

Hathaway (1997) designed risk-based HACCP plans in beef production in New Zealand. Beef production in New Zealand has characteristics typical of a temperature climate and pasture based animal husbandry. The specific pathogens which may contaminate fresh beef and which are empirically considered to be of public health importance are *Salmonella, Compylobacter,* and *E.coli0157:H7* and *Listeria monocytogens*. Almost all transmissions of these hazards, through consumption of beef results from unseen microbial cross-contamination from gastrointestinal source, during slaughter, dressing and further processing. Gaining comprehensive information on carcass contamination levels is an essential first step in established food safety objectives for a particular beef production system and designing risk-based HACCP plans.

Lara *et al*. (1997) applied HACCP in slaughterhouses in Spain. The critical points are to be considered as source of bacteriological contamination of carcasses in slaughterhouses. The manipulation and processing of carcasses in slaughterhouses reveals as a main source of microbiological pathogenic contamination affecting the carcasses.

Maria (1997) conducted HACCP system in sheep slaughtering operations at 4 different plants in Ireland and to determine the difference between plants in terms of microbial contamination. A single carcass area, the abdomen, was examined by swabbing and a microbiological profile was determined at different stages, along the slaughter line. The level of contamination was assessed from the total bacteria counts, Enterobacteriaceae and *Listeria* spp.

Park *et al*. (1997) applied HACCP principles in meat industry of United Kingdom. Because of the rising incidence of microbial foodborne disease in the UK, particular attention is being given to the application of HACCP principle in all sectors of the food industry including meat production. The basic requirements of HACCP system for the red meat and poultry industries are well known, but not yet uniformly applied in UK abattoirs. The use of HACCP system in the production of ready to eat meat and poultry products is considered essential for optimum pathogen control.

Tebutt *et al.* (1997) conducted HACCP for microbiological monitoring in cooked meat product plants. A combined risk assessment and microbiological sampling program was used to study compliance with recent food Legislation in cooked meat product plants. Eight key stages in production assessed were raw material, delivery and storage, raw material preparation, cooking, cooling, storage, meat slicing distribution and finally general areas relevant to the overall process. Meat product either, pies or cooked joints and a variety of environmental sites, including wiping cloths were identified. There was a strong link between microbial results from environmental sites and visual inspection in premises manufacturing pork pies, but not such association was found in those producing meat joints.

Buchanan *et al.* (1998) linked HACCP plans with public Health. The HACCP plan adoption has greatly enhanced the food industry's ability to systematically design programs to ensure microbiological safety of food. The integration of HACCP plans with the development of dynamic risk assessment model offers a mean for considering the entire farm to table continuum and for relating food-manufacturing operation to public health goals.

Freese *et al.* (1998) compared the microbiological quality of street food with food prepared at low income houses and at four or five star hotel restaurants in Guatemala City and Antigua. A total of 72 samples including a meat meal, a plant meal and staple meal were collected from all three food sources and analysed. Aerobic mesophilic count (APC), coliform and *E. coli* were used as microbial indicators. The highest count of colony forming units was obtained in meat meal than in others. For this meal, significantly ($P<0.05$) lower APC were found in house-prepared food than in street food and hotel prepared food, but there were no significant differences in coliform counts or *E. coli* counts among the sources of the meals. The analysis showed higher cfu counts for the components that were not heated, the avocado cream and tomato sauce, than for those

heated, grilled beef and tortillas. Significant differences were found between the heated and non-heated foods for APC ($P<0.01$) and coliforms count ($P<0.01$), but not for *E. coli.*

Maria *et al* (1998) conducted HACCP system to evaluate microbial hazards during processing of Spanish prepared flamenquin. Flamenquin is a traditional, prepared, frozen meat product of South Spain made with minced pork, chicken and cooked ham. Since it is a prepared raw meat product some microbial hazards could be associated with the process of making. Microbiological analysis has been performed throughout the various step of processing over 1 year period to evaluate microbial hazards in the commercial process. High levels of microorganisms were observed all through processing of this product, the mincing and mixing steps being where major microbial contamination was observed. Pathogenic bacteria such as *S. aureus*, *Cl. perfringens*, *E. coli*, and *Pseudomonas aeruginosa* were detected during processing. Raw materials and food handlers were the principle sources of microbial contamination. A modification of processing to include a heating step after mincing and mixing and improvement in hygienic practices could eliminate the microbial hazards. Both modifications should be noted for the implementation of HACCP program in commercial flamenquin processing.

Smith *et al.* (1998) applied HACCP principle on meat and poultry slaughtering and processing plants to establish new regulation, for microbiological testing criteria for *E. coli* and *Salmonella*, as a means of evaluating plant performance.

The HACCP subcommittee of the National Advisory Committee on Microbiological Criteria for Food, (NACMCF) (1998) has prepared a revision of the document HACCP system that was adapted by the committee in 1992. The committee retained the previous seven HACCP principles but made their wording more concise, revised and added definition such as those for hazard, verification and validation, including now section on prerequisite programs, education and training and implementation and maintenance of the HACCP plan, revised and provided a more detailed explanation of the application of HACCP principles, especially hazard analysis and verification

Blaha (1999) published a paper to describe the impact of the farm to table concept and the implementation of HACCP plans throughout the food production chain on animal production and veterinary profession, using the example of the pork production chain.

Chung (1999) applied HACCP system in the pork industry in Korea. The occurrence of food borne disease outbreaks are increasing in Korea. Among the outbreaks, *Salmonella, S. aureus, Vibrio parahaemolyticus* are the most important organisms and meat and meat products are the major sources of infection. The HACCP is a process control system designed to identify and prevent microbial and other hazards in food production.

Pedrso *et al*. (1999) determined Hazards and Critical Control Points (CCP) associated with meat balls and kibbe preparations in a hospital kitchen by using flow diagrams and microbiological testing of samples collected along the production line. Microbiological testing included count of mesophilic and psychrophilic microorganisms, yeasts and molds, total fecal coliforms, *Clostridium perfringens*, coagulase positive staphylococci, bacteria of the *Bacillus cereus* group and detection of *Salmonella*. Time/temperature binomial was measured in all steps of preparation. A decision tree was used to help in the determination of CCPs. The detected hazards were contamination of raw meat and vegetables, multiplication of the microorganisms during meat manipulation, poor hygiene of utensils and equipment and survival of microorganisms to the cooking process. Cooking and hot holding were considered CCPs. The results stress the importance of implementation of a training program for nutritionists and food handlers and the monitoring of CCPs and other measure to prevent foodborne diseases.

Anderson *et al.* (2000) used industry perspectives on the use of microbial data for HACCP. Validation and Verification of microbiological testing is an essential element in validation of critical limits identified within a HACCP Plan. Without appropriate validation there is no assurance that they have been validated to effectively prevent, reduce or eliminate hazards. Application of routine testing for pathogens in finished

product become an effective means to assure process control and also the safety of the product.

Cauto *et al.* (2000) developed quality audits of HACCP systems. The growing implantation in food industries of self-control systems based on the HACCP system; compel the hygiene inspection services to supervise these systems, to check if their design is appropriate to fulfill the objective of producing reliable food and observing their implantation into practice.

Fernando *et al*. (2000) applied HACCP in pork sausages industry of Mexico City. The presence of *Salmonella* spp. was studied as a part of HACCP in the process of three types of selected pork in Mexico City. Sample of five lots of pork sausages were analyzed (n=469). Samples were obtained during the production process (prime matter, matter process and finished product). Analysis included were sample of fecal matter (n=109), hands of personnel (n=118), in addition to inert surfaces (n=102) that were in direct contact with the raw materials in the process or in the finished product. The presence of *Salmonella* spp. in pork sausages is a hazard which represents a risk, and contributes to the HACCP application as a method to achieve the innocousens of three pork sausage preventing the public health problem.

K.C (2000) studied bacterial analysis of street food in relation to child health. Among the street food analysed, the coliform count in *momo* was found to be 2.4×10^2 cfu/gm. The different organisms isolated from *momo* were *Bacillus* spp., *E. coli, Klebsiella oxytoca, Micrococcus* spp., *S. aureus,* and *S. epidermidis.*

Peter *et al.* (2000) used application of foodborne disease outbreak data in the development and maintenance of HACCP systems. Five hundred and thirty general foodborne outbreaks of food poisoning reported in England and Wales between 1992 and 1996 were reviewed to study their application to the development and maintenance of HACCP systems. Retrospective investigations of food borne disease outbreaks provided

information on etiological agents, food vehicles and factors that contributed to the outbreaks, *Salmonella* spp. and foods of animal origin (red meat, poultry and sea food) were most frequently associated with outbreaks during this period. Improper cooking, inadequate heats, storage, cross contamination and use of raw ingredients in the preparation of food was the most common factors contributing to outbreaks.

Karmacharya *et al.* (2000) studied microbial evaluation of fresh and steamed *momo* frozen for different time intervals. During the study period, buff *momo*, mutton *momo*, chicken *momo* and pork *momo* were evaluated. Buff *momo* under fresh condition showed TPC/ gm to be 57×10^4, YMC/gm to be 18×10^1 and coliform /gm to be 4×10^3. *Salmonella* was found to be negative while *E. coli* was found to be +ve. Buff *momo* when steamed for 15 minutes showed TPC/ gm to be 3×10^2, while YMC, coliform; *Salmonella* and *E. coli* were all negative. Buff *momo* frozen (1 day) when evaluated showed TPC/gm to be 13×10^3, YMC/gm 5×10^1, coliform/gm 5×10^2 and *Salmonella* and *E. coli* were found to be negative. Chicken *momo* in fresh condition showed TPC/gm to be 60×10^3, YMC/gm was –ve, coliform/gm 40×10^3, while *Salmonella* and *E. coli* were found to be negative. Similarly, when steamed for 15 minutes gave TPC/gm to be 22×10^2 while YMC, coliform, *Salmonella* and *E. coli* were found to be negative. Chicken *momo* under frozen condition (22 days) showed TPC/gm to be 64×10^3, YMC/gm 4×10^1, coliform/gm 29×10^2 while *Salmonella* and *E.coli* were found to be negative.

Shrestha (2001) studied hazard analysis critical control point (HACCP) system in sausage production plants. Total aerobic mesophilic (TAMC) was ranged from 6×10^2 to 3.1×10^6 cfu/gm in industry A and 1.5×10^3 to 4×10^7 cfu/gm in industry B. Coliform count ranged from 0 to 5.8×10^5 cfu/gm in industry A and 0 to 2.6×10^6 cfu/gm in industry B. The yeast and mold count ranged from 0 to 7.5×10^3 cfu/gm in industry A while industry B had 0 to 6.9×10^6 cfu/gm.

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 MATERIALS

The materials used in this study are in the appendix II, IV and V.

4.2 STUDY METHODS

The main purpose of the study was to analyze the hazards associated with restaurant foods and determine its critical control points (CCPs). For the determination of CCPs, samples were collected from different stages of food preparation to determine the sources of contamination. Similarly the analysis was done in raw materials, final products and its subsequent stages. During the study, total aerobic mesophilic bacterial count, total coliform count, total staphylococcal count, total yeast and mold count was performed. In addition, presence of *Escherichia coli, Salmonella* spp. and *Staphylococcus aureus* was also performed.

4.3 SURVEILLANCE STUDY

The study was conducted in different restaurants of Kathmandu Metropolitan City (random sampling of restaurants) from April 2005 -Nov 2005. Field observation and interview with the owner and workers of the restaurant via structured questionnaire was carried out.

4.4 SAMPLES ANALYSED

Different preparation stages and final steamed product of buff *momo* and chicken *momo* were analysed.

4.5 SAMPLE COLLECTION

Different preparatory stages of buff *momo* and chicken *momo* (raw meat, mixture of minced meat with spices, raw *momo*, spices, pickles, flour, steamed *momo* and water) were collected aseptically with spoons or forceps which was cleaned and inserted into 95% alcohol and flamed. During sampling, temperature of the food was measured by inserting an ordinary mercury thermometer which was washed and immersed in 95% alcohol before inserting into the food. Time was subsequently observed. The samples were then collected in sterile plastic bags and placed immediately in an insulated container with ice and taken to the "Central Food Research Laboratory, Babar Mahal, Kathmandu" as soon as possible and processed on the same day or samples were frozen overnight and processed next day.

4.6 LABORATORY PROCEDURES

4.6.1 Preparation of food homogenate and serial dilution

a) 25 gram of sample was aseptically transferred into a sterile blender and 225 ml of sterile Buffered Peptone water (BPW) was added to it and blended for not more than 2.5 minutes. This will be 10^{-1} dilution.

b) The food homogenate was mixed well by shaking. 1 ml of food homogenate from 10^{-1} dilution was pipetted out aseptically into a tube containing 9 ml of sterile buffered peptone water (BPW) with the help of sterile pipette and carefully mixed by continuous shaking in a shaker and labeled as 10^{-2} dilution.

c) From the 10^{-2} dilution, 1 ml was transferred into a second dilution tube containing 9 ml of BPW, mixed well and labeled as 10^{-3} dilution.

d) Similarly, the process was repeated up to 10^{-6} dilution and labeled as 10^{-4}, 10^{-5}, and 10^{-6} dilution.

e) After each dilution, the tube was shaked well with the help of shaker to make a homogenate sample.

4.6.2 Enumeration of total aerobic mesophilic bacteria (Total bacterial count)

a. One ml from each dilution including food homogenate were pipetted out aseptically with the help of sterile pipette and kept into each of sterile appropriately marked duplicate plates.

b. Sterilized Plate Count Agar (PCA) was cooled down to 45°C and poured into each petridish within 15 minutes of the time of original dilution.

c. Sample dilution and agar medium were mixed thoroughly and uniformly. Then the plates were allowed to solidify.

4.6.3 Enumeration of coliform bacteria

a. One ml from each dilution including food homogenate was pipetted out and kept into each of sterile appropriately marked duplicate plates.

b. Sterilized Violet Red Bile Agar (VRBA) was cooled to 45°C and was poured into each plate within 15 minutes of the original dilution.

c. The sample dilution and agar medium were mixed thoroughly and uniformly. Then the plates were allowed to solidify.

4.6.4 Enumeration of yeast and mold

a. One ml from each dilution including food homogenate was pipetted out and kept into each of sterile appropriately marked duplicate plates.

b. Sterilized Potato Dextrose Agar (PDA) was cooled to 45°C and was poured into each plate within 15 minutes of the original dilution.
c. The sample dilution and agar medium were mixed thoroughly and uniformly. Then the plates were allowed to solidify.

4.6.5 Incubation

The inoculated petriplates containing PCA, VRBA were incubated at 37°C for 48 hours and plates with PDA were incubated at 28°C for 3-5 days.

4.6.6 Counting of colonies

Total bacterial counts were taken from the pour plates of Plate Count Agar and total coliform counts were taken from the pour plates of Violet Red Bile Agar. Similarly, total yeasts and mold counts were taken from the pour plates of Potato Dextrose Agar. Yeasts and molds count were reported per gram or ml. While counting, even the pinpoint colonies were counted. The petriplates containing 30-300 colonies after 48 hours incubation were counted.

4.6.7 Detection of *Escherichia coli*

4.6.7.1 Preparation of food homogenate dilution: Same as 4.6.1

4.6.7.2 Enrichment

a. A sterile pipette was taken and 1 ml of the food homogenate prepared during pour plate was transferred into 10 ml of sterile nutrient broth in a tube and incubated at 37°C for 24 hours.

4.6.7.3 Plating method

a. The culture was streaked on previously dried sterile Eosin Methylene Blue EMB) Agar with the help of sterile loop & incubated at 37°C for 24 hours.

b. Isolated colonies of 2-3 mm diameter exhibiting a greenish metallic sheen by reflected light and dark purple centre by transmitted light were further confirmed by morphological and biochemical tests

4.6.8 Detection of *Salmonella species*

4.6.8.1Preparation of food homogenate: Same as 4.6.1

4.6.8.2 Pre-enrichment

a. The food homogenate was transferred aseptically in sterile 500 ml conical flask and incubated at 37°C for 24 hours.

4.6.8.3 Enrichment

a. The incubated sample mixture was gently shaked.

b. 1 ml of the pre-enriched culture was transferred to 9ml of tetrathionate broth with the help of sterile pipette & incubated at 44°C for 48 hours.

4.6.8.4 Plating method

a. After incubation, the enriched culture was streaked on previously dried Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate (XLD) Agar with the help of sterile loop and incubated at 37°C for 24 hours.

b. The plates were then examined for typical colonies of *Salmonella*. Pink or red colonies surrounded by bright red medium on BGA and pink colonies

with or without black centers on XLD Agar were the main characters of salmonellae.

c. The typical colonies were identified by its morphological & biochemical tests.

4.6.9 Enumeration of *S. aureus*

4.6.9.1 Preparation of homogenate and serial dilution: Similar as in 4.6.1

4.6.9.2 Spread plating

a. 0.1ml of food homogenate and dilution of homogenate were pipetted out into the surface of previously dried Mannitol Salt Agar (MSA) plates and was spread with sterile bend glass rod on the surface of the medium. A duplicate plate was also prepared for each dilution.

4.6.9.3 Incubation

a. Plates were incubated at 37°C for 24-48 hours.

4.6.9.4 Counting of colonies

a. On MSA, presumptive coagulase positive staphylococci produced colonies with bright yellow zones.

b. Then the suspected colonies were further confirmed by morphological, coagulase and other diagnostic tests.

4.6.10 Pure culture for identification

After the enumeration of the organisms, each specific colony was chosen from VRBA and sub-cultures were made on Eosin Methylene Blue (EMB) Agar, Nutrient Agar (NA) and MacConkey Agar (MA) by streaking with the help of sterilized wire loop. Similarly, specific colonies from MSA were also chosen and subcultures were made on (NA) by streaking by means of sterilized wire loop. The streaked plates were then incubated at 37°C for 24-48 hours. So made subculture must be pure for the further identification of an unknown bacterial culture. Thus, obtained culture of organism on nutrient agar was used to perform catalase test, oxidase test and for Gram staining.

4.6.11 Plate exposure method for indoor air study

a. Nutrient agar plates and Blood agar plates were exposed for 15-20 minutes in the food serving area of the restaurant.
b. Those plates were incubated at 37°C for 24-48 hours.
c. Identification was done by colonial, morphological and biochemical characteristics.

4.6.12 Water quality test (MPN of drinking water)

4.6.12.1 Sampling of water from euro-guard and filter

a. The sterile BOD water bottle was taken.
b. For a few second, the water from the euro-guard and filter were made to flow.
c. The bottle was rinsed, filled and closed with the glass stopper and taken to lab in the ice box.

4.6.12.2 Presumptive test

a. Three sets of three groups Brilliant Green Lactose Bile Broth (2%) of ten ml each were taken and labeled as 3 single strength BGLB broth tubes "0.1", another 3tubes "1" and the three double strength broth tubes "10".
b. Aseptically double strength tubes were inoculated with the 10 ml test water using 10 ml sterile pipette. Similarly, each "1" labelled tubes were inoculated with 1 ml water using sterile pipette and the other "0.1" labelled tubes too were inoculated with 0.1 ml water using 0.1 ml sterile pipette.
c. All the inoculated tubes were incubated at 37°C for 24-48 hours.
d. All the tubes were examined for the production of acid and gas indicated by bubbles trapped inside (Durham's tube) after 24 hours.
e. Positive tubes were noted and again checked for the gas in 44°C(for fecal coliform test) and negative tubes were further incubated at 37°C for 24 hours and observed for acid and gas formation.

4.6.12.3 Confirmed test

a. From the gas positive tubes of both the temperatures, one loopful of the culture were streaked on the EMB plates.
b. The plates were incubated in an inverted position at 37°C for 24 hours.
c. Colour of colonies (typical, atypical and colourless) was noted.

4.6.12.4 Completed test

a. Isolated typical and atypical colonies from positive confirmed plates were inoculated in BGLB broth.
b. Similarly, isolated colonies from +ve confirmed plates were streaked in NA plates.
c. The plates and broth tubes were incubated at 37°C for 24 hours.
d. Gas formation were noted in case of BGLB broth and gram staining was performed from NA plates

4.6.12.5 Identification

a. Identification was done on the basis of biochemical tests.

4.6.13 Swabbing procedures

a. The hands were disinfected using 95% alcohol.
b. The utensils and equipments were swabbed with different sterile cotton buds dipped in sterile buffer peptone water and the cotton swab was kept in sterile tubes and transferred to the lab.
c. The swab was inoculated on the MSA to make the primary inoculum and with the help of different sterile loops, it was streaked.
d. The plates were incubated at 37°C for 24 hours.
e. The plates were then observed for the significant growth of colonies.
f. The isolated colonies were subcultured on NA.
g. Identification was done on the basis of biochemical tests.

4.6.14 Quality control for tests

Strict aseptic condition was maintained throughout the study. Quality of each test was maintained by using standard procedures. Sterility testing of each batch of culture and biochemical medium were checked by incubating one or two uninoculated tubes and plates of each lot with inoculated ones. Batch of the medium was discarded when uninoculated plates or tubes showed growth. During identification of organisms, for each test ATCC control positives and control negatives was taken simultaneously. Control test was also performed to confirm that the medium especially biochemical medium was made up correctly.

CHAPTER-V

5. RESULTS

5.1 SURVEILLANCE RESULTS

In the present study, survey was conducted among 8 restaurants in Kathmandu Metropolitan City with formatted questionnaire. During this survey, owners of the restaurants and the working personnels were interviewed randomly to know about their knowledge in the hygienic practices and the sanitary condition of the restaurant. Besides these, surveillance study also focused on their knowledge on micro-organisms and the health hazard associated with unhygienic practices.

The survey included the condition under which the owners operate, the way of food handling and the personal hygiene of the employees. In addition, purchasing or procurement of raw materials, the handling of the raw materials during preparation and during serving to the customers, the storage of food and partly prepared foods were also included in the study. Similarly, the environmental condition of the restaurant was studied by plate exposure method and swab of the chop-board, serving plate, mincer, storage vessel were also included as a part of the sanitation study. The necessary information was obtained by observing the preparation of food products and interviewing the chefs with the help of formatted questionnaire (Appendix I).

5.1.1 Results for knowledge of sanitation and health education

The analysis of survey result showed that 62% of the restaurants studied had trained staffs in sanitation while 38% of the restaurants had untrained staffs. In our survey result 25% of the restaurant had given health education to their staffs only in training period, 13% of the restaurant had given health education to their staffs when required and the rest had not trained working personnels in health education.

5.1.2 Results for water for preparation of foods, drinking and its storage

According to the survey report, it was found that 13% of the restaurant used “purified” mineral water, 25% used well water while 62% of the restaurant used water distributed from municipality pipeline for preparation of food. Water for preparation of food as well as drinking purpose was used from same source in 62% of the restaurant while 38% of the restaurants used different sources.

Similarly, 38% of the restaurant owner replied that water for washing purpose was treated while 62% replied that water was not treated. 33% of the restaurant used the boiling method for treatment of water, 17% used normal filtration method, 17% used ‘Euro-guard’ while 33% used filtered and then boiled water. Water for drinking as well as for preparation of foods was stored in plastic jar in 13% of the restaurant while 87% stored water PVC tank. The entire restaurant using ‘plastic jar’ for storage of water cleaned the storage vessel daily and the reason behind it was the convenience to wash the small ‘plastic jar’. The owners of all the restaurants using tank for storage of water replied that the tanks were washed monthly, but in our observation, some of the tanks did not found to be washed in a month at all.

5.1.3 Results for handling of cooking utensils and storage of knives and chop-boards

In the questionnaire asked during studies, all restaurant owners replied that the cooking utensils were cleaned before cooking, but it did not seem so in 38% of the restaurants. Similarly, all the restaurant owners replied that the knives, chop-boards were cleaned before and after cutting, but it was not found to be so in 62% of the restaurants. 87% of the restaurant owners replied that knives and chop-boards were stored in shelves while 13% said that they hang chop-board and knives. On observation 62% of the restaurants had these materials left on the table openly without proper cleaning which can be one of the reasons for cross contamination.

5.1.4 Results for food preparation, storage and handling practices

It was observed that unperishable raw materials were stored at room temperature while perishable items like, meat and other meat products such as mixture of minced meat with spices and pickles were stored in fridge. During survey, all of the restaurant owners replied that the raw materials were washed thoroughly before preparation, but in our observation, it was found that the meat stored in refrigerator was not cleaned before preparing the items. Before cooking all of the restaurants working personnels replied that they wash their hands but on the spot observation 62% of the restaurants personnels washed their hands with water only while 38% used soap as well before preparation of food.

In all of the restaurants, pickles and ready to steam *momo* were prepared beforehand and kept in the fridge till order came. The longevity of storage of prepared foods varied among the restaurants. 62% of the restaurant owners replied that the cooked foods were stored for certain hours, 25% stored all day long depending upon the type of season while 13% stored until sold. According to our surveillance study, 50% of the restaurant owners replied that the food items were sold on the same day while 50% said that the food items could not be sold on the same day. 25% of the restaurant owner replied that they kept the left over foods for next day in refrigerator and deep fridge, 50% replied that they threw it away which also depended upon the type of season and the nature of food material while 25% said that they ate it up at the end of the day.

5.1.5 Results for serving and packaging practices

87% of the restaurants used spoon and other serving utensils while serving of food while 13% used both spoon and hand. But hand washing practices before serving of the food was not observed at all.

The used plates were washed immediately in all of the restaurants. It was also found that 62% of the restaurants surveyed used soap for washing, 25% used detergent and 13% used both soap and detergent for the washing purpose. Similarly, the packaging material for home-pack system was found to be aluminium foil in all of the restaurants surveyed.

5.1.6 Results for personal hygiene and environmental condition

In our study, 62% of the working personnels seemed to take care about their personal hygiene while 38% did not do that. Since, the study was conducted in Kathmandu Metropolitan City; it is no doubt that the environment is polluted and crowded. But, we also observed the type of location of the restaurants which upon observation, 74% of the restaurants surveyed were in crowded area, 13% were in fair area while 13% of them were found to be dusty and crowded area.

5.2 Microbiological quality of the air environment inside the different restaurants of the Kathmandu metropolitan city

Table 1. Air quality assessment of the serving region in the restaurants

Restaurant of	Types of organisms obtained
Site A (Baneswor)	*Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp.
Site B (Kalanki)	*B.* spp., *S.* spp., *M.* spp., *Pseudomonas* spp.
Site C (Chabahil)	*B.* spp., *S.* spp., *M.* spp., *Ps.* spp.
Site D (Khichapokhari)	*B.* spp., *S.* spp., *M.* spp., *Ps.* spp.
Site E (Thamel)	*B.* spp., *S.* spp., *M.* spp.
Site F (Maharajgunj)	*B.* spp., *M.* spp., *S.* spp.
Site G (Balaju)	*B.* spp., *M.* spp., *S.* spp., *Ps.* spp.
Site H (Bhatbhateni)	*B.* spp., *M.* spp., *S.* spp.

The Table 1 represents the internal air quality of the different restaurants which shows the types of organisms obtained. The restaurant environment was found to be more or less similar with *Bacillus* spp., *Micrococcus* spp. and *Staphylococcus* spp. in descending order.

5.3 Drinking water assessment of different restaurants

Table 2. MPN of drinking water

Restaurants of	MPN per gm or ml	Coliform	Fecal coliform
Site A (Baneswor)	<3	-	-
Site B (Kalanki)	<3	-	-
Site C (Chabahil)	<3	-	-
Site D(Khichapokhari)	>2,400	+	+
Site E (Thamel)	<3	-	-
Site F (Maharajgunj)	<3	-	-
Site G (Balaju)	15	+	-
Site H (Bhatbhateni)	<3	-	-

The result of MPN of drinking water showed the restaurant of site D had fecal coliform contamination and the restaurant of site G had the coliform contamination in drinking water while the rest of the restaurants had no coliform.

5.4 Sanitary survey result

The sanitary survey result of the utensils and equipments (storing vessel of meat, serving plate, meat mincer and chop-board) analysed for *S. aureus* showed the positive result from all of the restaurants.

5.5 Microbiological assessment in different stages of "Chicken *Momo*" and "Buff *Momo*" preparation process

During the study, total 8 restaurants of the Katmandu Metropolitan City were randomly selected and from each restaurant, raw ingredients to the final steamed "chicken *momo*" and "buff *momo*" as well as the pickles served with that cuisine was analyzed for total aerobic mesophilic count, coliform count, *S. aureus* count, *Salmonella species* count, *E. coli* count, yeasts and molds count.

Samples were collected at the different stages of chicken *momo* and buff *momo* preparations. The samples included for chicken *momo* are:

Sample P1= Raw chicken meat
Sample P2= Mixture of minced chicken with spices
Sample P3= Raw chicken *momo*
Sample P4 = Steamed chicken *momo*
Sample P5 = Pickles
Sample P6 = Flour
Sample P7= Mixture of spices

The different samples taken from "Buff *momo*" preparation are:
Sample P9= Raw buff meat
Sample P10= Mixture of minced buff with spices
Sample P11= Raw buff *momo*
Sample P12 = Steamed buff *momo*
Sample P13 = Pickles
Sample P14 = Flour
Sample P15= Mixture of spices

5.6 Identification of pathogenic micro-organisms

Table 3. Identification of *Salmonella species*, *Escherichia coli*, Coagulase positive *Staphylococcus aureus* in different stages of "Chicken *Momo*" preparation

Restaurant of	No. of samples	*Salmonella* spp.		*Escherichia coli*		Coagulase positive *Staphylococcus aureus*	
		+ve	%	+ve	%	+ve	%
Site A	7	0	0	0	0	4	57
Site B	7	0	0	4	57	5	71
Site C	7	0	0	5	71	5	71
Site D	7	0	0	4	57	6	86
Site E	7	0	0	0	0	4	57
Site F	7	0	0	0	0	4	57
Site G	7	0	0	5	71	6	86
Site H	7	0	0	0	0	4	57

All of the samples of chicken momo from different restaurants analysed during the studies didn't show the presence of *Salmonella* spp.

However, the samples analysed for the presence of *E. coli* showed the result that in restaurant of site B, 57% of the samples were contaminated with *E. coli* and the samples were P1, P2, P3 and P7. Similarly, restaurant of site D also had the same result while restaurants of site C and G had 71% of *E. coli* contamination in the samples P1, P2, P3, P6 and P7. But the restaurants of site A, E, F and H did not show the presence of *E. coli* in any of the samples.

In restaurants of site A, E, F and H, the coagulase positive *S. aureus* isolated were 57% of the total samples. Similarly, in restaurants of site B and C the coagulase positive *S. aureus* isolated were 71% of the total samples while in restaurant of site D and G, the coagulase positive *S. aureus* isolated were 86% of the total samples.

Table 4. Identification of *Salmonella species*, *Escherichia coli*, and Coagulase positive *Staphylococcus aureus* in different stages of "Buff *Momo*" preparation

Restaurant of	No. of samples	*Salmonella* spp.		*Escherichia coli*		Coagulase positive *Staphylococcus aureus*	
		+ve	%	+ve	%	+ve	%
Site A	7	0	0	0	0	4	57
Site B	7	0	0	4	57	5	71
Site C	7	0	0	5	71	5	71
Site D	7	0	0	4	57	5	71
Site E	7	0	0	0	0	4	57
Site F	7	0	0	0	0	4	57
Site G	7	0	0	5	71	6	86
Site H	7	0	0	0	0	4	57

All of the samples of buff momo from different restaurants analysed during the studies didn't show the presence of *Salmonella* spp.

However, the samples analysed for the presence of *E. coli* showed the result that in restaurant of site B, 57% of the samples were contaminated with *E. coli* and the samples were P9, P10, P11 and P15.Similarly, restaurant of site D also showed the same result while restaurants of site C and G had 71% of *E. coli* contamination in the samples P9, P10, P11, P14 and P15. But the restaurants of site A, E, F and H did not show the presence of *E. coli* in any of the samples.

In the restaurants of site A, E, F and H, the coagulase positive *S. aureus* isolated were 57% of the total samples. Similarly, in restaurants of site B, C and D the coagulase positive *S. aureus* isolated were 71% of the total samples while in restaurant of site G, the coagulase positive *S. aureus* isolated were 86% of the total samples.

The table 5 showed that the effective Critical Control Points (CCP) is steaming where the microbial hazards can be reduced to completely safe level. Other Control Points (CPs) are not the effective control points, however, can minimize the microbial loads. The pathogenic microbes were killed during steaming of the *momo* for 15-20 minutes at 75°C-80°C. So the steamed *momo* won't get contaminated until and unless served in clean serving plate with spoons and forks.

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Momo is a meat based product and the consumption of *momo* is increasing as a snack in urban areas. Meat and meat based foods are regarded as of high nutritive value due to the concentrated sources of protein and some of the vitamins of B complex group. Due to this nutritive value, growth of microorganisms including many pathogens is favoured on meat and meat based products. These microorganisms may enter into the food products from raw materials, water, unclean cooking utensils, and environmental contamination and by the person involved in the preparation of food and sale. Depending upon the types of microorganisms introduced into the food, they multiply rapidly and reach the levels sufficient to produce infections or intoxication (Frazier and Westhoff, 2001).

The number of organisms in a particular food substance at any given time depends on the nature of food stuff, its temperature and the duration of the time it has been kept. Similarly, the contaminating organisms may include microorganisms responsible for food-borne illness, but the number or dose of organisms necessary to infect or to produce sufficient toxin to cause symptoms varies with different organisms, and also varies with the immunity of the person who ingested the food. Although the microbial population in the food stuff does not cause illness, certain microbial contamination is an indicator of poor sanitary practice in the preparation and storage of the food (Banwart, 1996).

It is estimated that upto 30% of the people in the industrialized countries may suffer from food borne illness each year. In the United States of America (USA), around 76 million cases of foodborne diseases, resulting in 3,25,000 hospitalization and 5,000 deaths cases are estimated to occur each year (WHO, 2000). Hence, the safety of the food and the role of microbes in the health hazard can not be ignored and overlooked.

In the context of Nepal, such epidemic associated with meat based products has not yet been reported which may be due to the lack of studies in this field. Moreover, the prevalence of foodborne diseases like gastroenteritis, typhoid (Salmonellae infection), bacillary dysentery (Shigellosis), cholera, jaundice, (Staphylococcal infection) and other diarrhoeal diseases can not be overlooked.

The work described in this study was undertaken in different restaurants of Kathmandu Metropolitan City to investigate the bacteriological quality and the safety of the fast food (*momo*) as well as the sanitary practice of those restaurants. During the study period, surveillance study was conducted in 8 different fast food restaurants of Kathmandu Metropolitan City with structured questionnaire and field observation.

On entering a food establishment, customer should get an impression of a bright, clean and attractive eating place so that they would revisit the place. Similarly the physical appearance and environmental condition of the restaurants surveyed seemed to be hygienic in most of the cases. But since Kathmandu City is much crowded, the locations of the 74% restaurants surveyed were in crowded area, 13% in fair place and 13% was found to be in dusty and crowded area.

Although the restaurants owners had knowledge about health education and sanitation, 38% of the restaurants had untrained staffs. It was also seen that the working staffs though knew about the consequences of improper sanitary practices, neglected to apply during their work. So health hazard issues due to consumption of unhygienic foods should be well understood by these employees too.

Clean and potable water should be used for preparation of foods and drinking purpose. So supplies have to be drawn from the best available sources. According to the survey result, majority of the restaurants had provided clean water for drinking as well as for preparation of dishes. 13% of the restaurant surveyed had provided the water contaminated with fecal coliform while 13% had supplied water containing coliform.

Although these restaurants had serviced the municipality supplied water, the treatment process did not seem to be the reliable one.

According to Maharjan (1993), the drinking water of Kathmandu is considered to harbour higher number of coliforms. Thus, water can be the source of coliform in foods. So in our study, the higher number of coliforms found in foods of the restaurant of site D, restaurant of site G must have made their way through the water used during preparation of the food.

Though the employees of all the restaurants were aware about basic sanitation, they ignored to apply practically. In 62% of the restaurants, the cutting utensils were left in the table after use without proper cleaning. This also can be one of the possible reasons for cross contamination which must be prevented by properly storing the products away from raw meat and soiled equipment and utensils. It was also observed that hand washing practices before serving of foods was not observed at all which can be one of the major reasons for contaminating meat based products with microorganisms like *S. aureus, E. coli* and other coliforms.

The cleanliness of utensils and equipments used in the restaurants were assessed to check the standard of hygiene and the efficiency of cleaning procedures. The study gives an idea whether the soaps and the detergents used are of good quality or having anti-microbial activity. Besides this, it also depends on the quality of water used for the washing. Hence, in our study it was found that these utensils and equipments were contaminated with *S. aureus* which must be due to the handling practices.

The survey result also showed that the unperishable raw materials were stored at room temperature while cooked and perishable food items were stored in refrigerated temperature. Separating raw foods from ready to eat products in the operation's refrigeration and storage facilities can control the potential for cross-contamination (FDA, 2005). But during serving time, the large volumes of cooked pickles were

displayed without giving any protection at ambient temperature until sold. This kind of activities can be a significant factor contributing to outbreaks of food-borne diseases due to the outgrowth of bacteria. Moreover, small batch preparation is an important tool for controlling bacterial growth because limiting the amount of food prepared minimizes the time the food is kept at a temperature that allows for the growth.

Personal hygiene of the working personnels should be considered as one of the important factor to determine the risk of cross contamination because these employees are the possible high risk group for cross contamination of edible materials if they have got lesions or open wound on their hand as well as if their aprons are dirty, the risk of contamination of food materials with the organisms like *S. aureus* is too high (Longree and Armbruster, 1996). But in our study, majority of the working personnels seemed to care about their personal hygiene.

Human beings harbour various pathogenic, nonpathogenic and opportunistic organisms on their skin, respiratory tract, intestinal canal, nasal cavity, hairs etc. During sneezing, coughing, scrubbing various microorganisms enter into the air environment (Katyal and Satake, 2001).

Obviously in any restaurant, the movement of the people is very high and therefore the chance of indoor pollution due to the movement of people is also one of the factors. So in order to monitor the indoor air quality, the plate exposure method was applied, which showed the presence of microorganisms like *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Pseudomonas* spp. in descending order.

The main purpose of this study was to investigate microbiological quality of “chicken *momo*” and ‘buff *momo*” from different restaurants of the Kathmandu City through Hazard analysis critical control point (HACCP) system. There is an increasing understanding that the microbiological quality of a certain food is the result of a chain of events. The microbial safety can be guaranteed when the overall processing and handling

are taken into consideration. Therefore, the microbiological quality assurance of food is not only a matter of control, but also of a careful design of the total process. Thus, to upgrade the hygienic condition of such eating places, hazards were analysed to determine the critical control points (CCPs) at which if control measures are applied carefully may upgrade the quality of such products.

Any food to be of good quality and safe for public health should be free from hazardous microorganisms and if present should be at safe level. So in order to fulfill this requirement in this study the total aerobic mesophilic count, total coliform count, total yeast and mold count were performed along with the detection of some target pathogens like *E. coli, Salmonella* spp., *S. aureus* from samples taken from different preparation steps of chicken *momo* and buff *momo*.

Unsanitary condition of food storage and handling inoculates large number of microbes to it. Various workers suggest that human being shed 10^3-10^4 viable organisms per minutes (Frazier, 1978 and Jay, 1987). The number and types of organisms shed is closely related to working environment.

In all of the restaurants, during microbiological assessment in different stages of chicken *momo* preparation, the sample P4 (Steamed chicken *momo*) showed insignificant total aerobic mesophilic count but did not show any yeasts and molds count, coliform count and staphylococcal count which may be due to the adequate steaming time and temperature required to kill all the vegetative cells and spores. So the result showed that the final steamed product is safe for human consumption until and unless the serving plate and handling practices is good.

The aerobic mesophilic count is useful for indicating the overall microbiological quality of a product and thus, is useful for indicating potential spoilage in perishable products. It also indicates the microbial survival following certain processing methods, or growth at CCPs, or the shelf life of a food. It is also useful for indicating the sanitary conditions

under which the food was produced and/or processed. High aerobic mesophilic count indicates that the food supports microbial growth, particularly in samples taken subsequentially.

The raw meat (Sample P1) was found to be highly contaminated in restaurant of site G (1.8×10^6) cfu/gm then followed by restaurant of site D (1.72×10^6) cfu/gm and lowest in restaurant of site H (6.5×10^5) cfu/gm. Sample P2 was found to be highly contaminated in restaurant of site D (2.2×10^6) cfu/gm while restaurant of site E had low count of (1.2×10^6) cfu/gm. The high count in sample P2 might be due to mixing of spices with the minced meat through not properly washed hands. The sample P3 showed that the restaurant of site B had elevated aerobic mesophilic plate count of (2.6×10^6) cfu/gm followed by restaurant of site D (2.5×10^6) cfu/gm and the low count in restaurant of site H (1.56×10^6) cfu/gm. From the results obtained, the sample P5 was found to be heavily contaminated in restaurant of site G (2.8×10^6) cfu/gm and less contaminated in restaurant of site H (6.8×10^5) cfu/gm as pickles were prepared beforehand in huge amount and stored in refrigerator but once the customer entered the restaurant and ordered the food, the pickles was reheated and displayed in serving area in room temperature until sold. Sample P6 was found to be heavily contaminated in restaurant of site B (2.34×10^6) cfu/gm and less contaminated in restaurant of site H (5.5×10^4) cfu/gm. Sample P7 contained (2.8×10^6) cfu/gm level in restaurant of site B while restaurant of site E had low level of (1.25×10^6) cfu/gm.

High count of yeast and molds indicate the environmental condition or sanitary condition of the processing, storing and distribution. It also reflects the presence of low pH, oxygen and moisturing condition of the processing or products.

Yeast and mold count was detected in high amount in sample P1 in restaurant of site C (1.13×10^3) cfu/gm while restaurant of site H had low count of (2.2×10^2) cfu/gm. Similarly, sample P2 showed high count in restaurant of site B and G with (1.32×10^3) cfu/gm and (1.3×10^3) cfu/gm respectively while restaurant of site H had low count of (3

$\times 10^2$) cfu/gm. Restaurant of site D and G had high count of (1.6×10^3) cfu/gm each in sample P3 while restaurant of site H had low count of (4×10^2) cfu/gm. Restaurant of site D had high count of (2.1×10^3) cfu/gm in sample P5 while restaurant of site E had low count of (3×10^2) cfu/gm. Sample P6 had high count in restaurant of site B (1.002×10^3) cfu/gm while restaurant of site F had low count of 2 cfu/gm. Sample P7 had low count in restaurant of site A (1.01×10^3) cfu/gm while restaurant of site F had high count of (1.55×10^3) cfu/gm.

Members of the total coliform and faecal coliform groups are referred to as indicator organisms since contamination of their presence is used to indicate the potential presence of pathogens in foods. The higher the number of coliforms, the greater the possibilities of pathogenic organisms present (FAO, 1992). Coliforms are used as an indicator of post process contamination of food.

Most of the samples in all the restaurants were contaminated with coliforms. Sample P1 was highly contaminated with total coliform count of (1.3×10^5) cfu/gm in restaurant of site D while less count of (2.5×10^4) cfu/gm in the restaurant of site F. Similarly, restaurant of site A had low count of (6.2×10^4) cfu/gm in sample P2 and (8.2×10^4) cfu/gm in sample P3 while restaurant of site D had highest coliform count of (1.6×10^5) cfu/gm in sample P2 and restaurant of site B had highest coliform count of (1.92×10^5) cfu/gm in sample P3. Restaurant of site E showed low count of (5.5×10^3) cfu/gm in sample P5 and no coliform count in sample P6 while restaurant of site G had the highest coliform count of (1.4×10^5) cfu/gm and (5.8×10^3) cfu/gm in sample P5 and P6 respectively. Similarly, restaurant of site G showed the highest coliform count of (1.03×10^5) cfu/gm in sample P7 while restaurant of site C had low coliform count of (2.2×10^3) cfu/gm.

Presence of *S. aureus* doesn’t indicate faecal contamination but indicates post processing contamination by persons who handled food. They are habituated in warm, damp and congenital atmosphere of the nose, throat, in the pores and hair follicles of the skin and

on the surface of skin. It is not easy to eliminate staphylococci from skin or nose. But washing hands with soap or similar substance may decrease the count to substantial level. High counts indicate that growth has occurred and possible presence of enterotoxins; thus can indicate a potential health hazard.

The staphylococcal count in sample P1 was found to be the highest in restaurant of site B and G having count of (2×10^3) cfu/gm while restaurant of site A showed the less count of (1.1×10^3) cfu/gm. Again the restaurant of site B showed the highest staphylococcal count in all the samples taken while restaurant of site H, F and E showed low staphylococcal count of (1.2×10^3) cfu/gm, (1.45×10^3) cfu/gm and (1.1×10^3) cfu/gm in sample P2, P3 and P5 respectively. Restaurant of site E and F didn't show any staphylococcal count in sample P6 while restaurant of site A showed the low count of (1.0×10^3) cfu/gm in sample P7.

Coagulase positive staphylococci isolated from food should be regarded as potential food poisoning types. The finding of a small number of staphylococci in a food is not necessarily significant even if some of them are coagulase positive. However, the finding of large numbers should cast suspicion on the handling, sanitation, and temperature of holding of the product. Any food product having upward of half a million coagulase-positive staphylococci per gram should be considered a public health hazard (Sharf, 1996).

There are many examples of outbreaks of Staphylococcal food poisoning caused by consuming food contaminated by *S. aureus*. They are transmitted through boils, ulcers, and abrasions on the hands and also from healthy looking hands. Therefore, care of hands should be done by washing before, between and after touching foods.
Salmonella spp. detection indicates presence of Salmonellae, cause of gastroenteritis and enteric fever. On heat-processed foods, presence of *Salmonella* spp. indicates survival or contamination after processing and cross-contamination. Presence of *Salmonella* not only indicates contamination of food with faeces of infected person but also indicates

contamination through excreta of animal like dog or hen. The intestine of these animals is as conductive for the survival and multiplication of Salmonellae as that of man.

All of the samples analysed were not contaminated by *Salmonella* spp.

E. coli is the best available indicator of possible faecal contamination, hence risk of presence of enteric pathogens and potential health hazard. It also indicates contamination after processing or process failure. *E. coli* can multiply in food and that large numbers of organisms (e.g. 10^5-10^7 organisms/gm) need to be present to cause infection (Eley, 1996).

Restaurant of site A, E, F and H did not show the presence of *E. coli* in any of the samples while in restaurant of site B, *E. coli* was isolated from the sample P1, P2, P3 and P7. In restaurant of site C, sample P1, P2, P3, P6 and P7 were contaminated with *E. coli*. Similarly, in restaurant of site D, sample P1, P2, P3 and P7 were contaminated by *E. coli*. Sample P1, P2, P3, P6 and P7 of restaurant of site G too was contaminated from *E. coli*.

The microbiological assessment of buff momo preparation in all of the restaurants showed that the sample H12 (Steamed buff *momo*) did not show any kind of yeast and mold count, coliform count and staphylococcal count however showed very insignificant total aerobic mesophilic count.

Sample P9 of restaurant of site B showed elevated total aerobic mesophilic count of (1.97×10^6) cfu/gm while restaurant of site H showed low total aerobic mesophilic count of (8.5×10^5) cfu/gm. Restaurant of site D and G had elevated total aerobic mesophilic count of (2.3×10^6) cfu/gm each in sample P10 and restaurant of site E had low count of (1.36×10^6) cfu/gm. Restaurant of site G had (2.8×10^6) cfu/gm in sample P11 and restaurant of site H had low count of (1.6×10^6) cfu/gm. Similarly, restaurant of site H had low total aerobic mesophilic count in rest of the samples while restaurant of site G had high count of (2.6×10^6) cfu/gm in sample P13 and restaurant of site B had high

count of (2.44×10^6) cfu/gm and (2.82×10^6) cfu/gm in sample P14 and P15 respectively.

Sample P9 had the highest yeast and mold count of (1.36×10^3) cfu/gm in restaurant of site D and (1.3×10^3) cfu/gm in restaurant of site G and lowest count of (4×10^2) cfu/gm in restaurant of site H. Sample P10 had low count of (5.2×10^2) cfu/gm in restaurant of site H and high count of (1.8×10^3) cfu/gm in restaurant of site G. Similarly, sample P11 had high count of (1.8×10^3) cfu/gm in restaurant of site G while low count of (1.2×10^3) cfu/gm in restaurants of site A, F and H. Sample P13 had high yeast and mold count of (1.9×10^3) cfu/gm in restaurant of site D and low count with (4×10^2) cfu/gm in restaurant of site H. Restaurant of site B had high count of (1.006×10^3) cfu/gm in sample P14 while low count with 4cfu/gm in restaurant of site H. Sample P15 had high count of (1.7×10^3) cfu/gm in restaurant of site F while low count with (1.04×10^3) cfu/gm in restaurant of site A.

The sample P9 showed the highest coliform count of (1.42×10^5) cfu/gm in restaurant of site B and low count of (3.1×10^4) cfu/gm in restaurant of site F. Sample P10 had the highest count of (1.6×10^5) cfu/gm in restaurant of site B and low count of (8.2×10^4) cfu/gm in restaurant of site A. Sample P11 had the highest count of (2.1×10^5) cfu/gm in restaurant of site D and low count of (1.18×10^5) cfu/gm in restaurant of site F. Similarly, sample P13 showed the high count of (1.42×10^5) cfu/gm in restaurant of site D and low count of 96cfu/gm in restaurant of site E. Sample P14 did not show any coliform count in restaurant of site E while high count of (6.3×10^3) cfu/gm in restaurant of site G. Sample P15 had high count of (1.02×10^5) cfu/gm in restaurant of site G and low count of (2.6×10^3) cfu/gm in restaurant of site C.

Sample P9 had high *S. aureus* count of (2.2×10^3) cfu/gm in restaurant of site D and low count of (8×10^2) cfu/gm in restaurant of site E. Sample P10 had high count of (2.3×10^3) cfu/gm in restaurant of site D and G and low count of (1.2×10^3) cfu/gm in restaurant of site E. Sample P11 had low count of (1.4×10^3) cfu/gm in restaurant of site

H and high count of (2.8×10^3) cfu/gm in restaurant of site C. Sample P13 had high count of (2.8×10^3) cfu/gm in restaurant of site B and low count of (1.3×10^3) cfu/gm in restaurant of site H. Sample P14 had high count of (2.2×10^3) cfu/gm in restaurant of site B and restaurant of site E and F did not show any growth of *S. aureus* in sample P6.Similarly, restaurant of site B had high count of (2.4×10^3) cfu/gm in sample P15 and low count of (1.2×10^3) cfu/gm in restaurant of site A.

The samples analysed were not contaminated by *Salmonella* spp.

Restaurants of site A, E, F and H did not show the presence of *E. coli* in any of the samples while in restaurant of site B, *E. coli* was isolated from the sample P9, P10, P11 and P15. In restaurant of site C, sample P9, P10, P11, P14 and P15 were contaminated with *E. coli*. Similarly, in restaurant of site D, sample P9, P10, P11 and P15 were contaminated by *E. coli*. Sample P9, P10, P11, P14 and P15 of restaurant of site G too was contaminated with *E. coli*.

The result obtained from the microbiological analysis assessed in seven key stages (raw meat, mixture of minced meat with spices, raw *momo*, steamed *momo*, pickles, flour, and mixture of spices) showed that, a considerably high levels of micro-organisms were observed throughout the various steps of both types of *momo* preparation process. The raw *momo* (sample P3 and P11) step being the major step, the highest microbial counts were observed. This is because the physical interventions like handling, cutting and mincing are even more serious and while mincing the contaminating bacteria on the surface are quickly dispersed through the whole mass. Moreover, the mincing process contributes to an increase in temperature which promotes faster bacterial growth. Similarly, the raw *momo* (sampleP3 and P11) is prepared with hands by mixing of minced meat with spices which can be the reason for the highest microbial count in raw meat and mixture of minced meat with spices.

After steaming, the quantitative results showed insignificant total plate count in both types of *momo*. Similarly, after steaming, the yeast and mold count, coliform count, *S. aureus* count were too reduced to 0cfu/gm. This indicated that the time/temperature exposure for steaming (75°C-80°C) for 15-20 minutes was sufficient to reduce or kill the micro-organisms. Thus from the above studies, it can be concluded that the steaming/cooking is the CCP at which steamed or cooked for sufficient period of time-temperature can reduce the coliform organism to the levels independent of the quality of raw materials. Thus, if cooking is done for sufficient period of time-temperature, it fully eliminates the harmful microbes.

The study also showed that samples P5 and P13 (pickles) were found to be heavily contaminated. The pickles made of tomato were prepared early in the morning in huge amount and were stored at refrigerator but during the lunch hour, it was displayed in the serving zone until finished without any protection which can be the possible reason for higher bacterial count. Moreover, when food is held, cooled, and reheated in a food establishment there is an increased risk from contamination caused by personnel, equipment, procedures, or other factors. So small batch preparation is an important tool for controlling bacterial growth because limiting the amount of food prepared minimizes the time the food is kept at a temperature that allows for growth.

Similarly, sample P7 and P15 (mixture of spices) too showed higher bacterial count because most of the grinder used for making the paste of spices were rarely cleaned until the end of the day after use and the paste of spices were touched with unclean hands too.

Comparatively, the microbial count of restaurants of site A, E, F and H were found to be lower than the restaurants of site B, C, D and G. This was due to the fact that, sanitary condition, personal hygiene of the employees and the handling practices were not quite good in restaurants of site B, C, D and G. The raw materials used for momo preparation were also not of higher quality in those restaurants.

Similarly, Joshi *et al*. (2000) conducted microbiological analysis of raw, ready to steam (RTS) and steamed *momo* whose study supports our findings that in all the samples, microbial load was found to be in descending order from raw, ready to steam and steamed *momo*. Though, the raw meat and the ready to steam momo contained some pathogenic bacteria (*E. coli*, *Staphylococcus* and *Salmonella*) they were all destroyed during steaming.

The findings of this study contradicts with the results obtained by Joshi *et al*.(2000) who examined 20 samples of raw *momo* and 27 samples of steamed *momo*. Total mesophilic counts were ranged between 10^2-10^6 cfu/gm of sample. Coliforms were present in 19 samples of raw *momo*, 11 of cooked *momo* and 1 of vegetable *momo*. *E. coli* was detected in 8 samples of raw *momo* only, 7 of cooked *momo* and 2 of vegetable *momo*. *S. aureus* was detected in 10 samples of raw *momo* and 1 of steamed *momo*.

K.C (2000) studied bacterial analysis of street food in relation to child health. Among the street food analysed, the coliform count in *momo* was found to be 2.4×10^2 cfu/gm. The different organisms isolated from *momo* were *Bacillus* spp*, E. coli, Klebsiella oxytoca, Micrococcus* spp.*, S. aureus,* and *S. epidermidis.*

Joshi (1996) studied the microbiological quality of traditional Nepalese meat based street foods and found that they were of poor hygienic quality. The total microbial load of final products of Kachila was found high upto 4×10^6 cfu/gm, Chhoyala upto 2.95×10^6 cfu/gm, Wo of black gram with meat upto 9.75×10^6 cfu/gm, Wo of green gram with meat upto 3.2×10^6 cfu/gm and Sekuwa upto 5.7×10^4 cfu/gm. The maximum total coliform counts were respectively 4.1×10^5 cfu/gm, 2.5×10^6 cfu/gm, 3.5×10^5 cfu/gm, 3×10^3 cfu/gm and 2×10^4 cfu/gm. Similarly, the maximum total *S. aureus* count was respectively 7.5×10^5 cfu/gm, 6×10^4 cfu/gm, 1×10^3 cfu/gm, 2.6×10^2 cfu/gm and 2×10^3 cfu/gm. The maximum total yeast counts were respectively 1.66×10^6 cfu/gm, 2.2×10^5 cfu/gm, 1.3×10^4 cfu/gm, 3×10^3 cfu/gm and 0 cfu/gm. The maximum total mold counts were 4×10^5 cfu/gm, 6×10^5 cfu/gm, 5×10^4 cfu/gm, 3×10^5 cfu/gm and 1×10^2 cfu/gm.

Shrestha *et al*. (1999) analysed 225 ethnic Newari meat varieties from Kathmandu valley restaurants (Sukuti, Kachila, Chooyala, *Momo*, Takha and Sanya khuna) were analysed for its hygienic quality. They found that 58% of the samples were contaminated with coliform, 1.8% with *S. aureus* and 3.6% with *Salmonella*. Total plate count varied from 10^1-10^5 cfu/gm. Out of 30 samples of Sukuti (Dry meat), 13% had coliform and 3% *Salmonella*. Out of 42 samples of Kachila 95% had coliform, 5% had *S. aureus* and 5% had *Salmonella*. Similarly out of 51 samples of Chhoyala 60% of samples had coliform, 2% had *Salmonella*. Out of 29 steamed *momo* samples, 17% saved coliform with *S. aureus* and *Salmonella* were absent.

Toppo *et al*. (1999) analyzed the level of bacterial contamination of pork luncheon meat at different stages of its production. Total viable count of cured and minced pork, as expressed in log 10 scale ranged from 6.3802 to 7.9912 with an average of 7.2987/gm. All the samples were positive for fecal coliform and the count varied between 3.3802 to 4.9777 with an average of 3.9805/gm. There was significant reduction in total viable count and coliform counts when the minced pork was subjected to heat treatment, during the preparation of pork luncheon meat. However, significant increase in total viable, coliform in sliced and packaged pork luncheon meat was observed due to post processing contamination.

Ganul *et al.* (1996) analyzed the microbiological quality of delicatessen food. A total 100 samples of delicatessen food products, which contained mayonnaise of yoghurt, salads with chicken, sausage other kinds of meats and vegetables. The analysis of total aerobic bacteria, coliform and fecal coliform, *E. coli, S. aureus*, and *Salmonella* were carried out on all samples. It was found that of 100 samples, 15% contained coagulase positive *S. aureus,* 79% contained coliform bacteria, 40% contained fecal coliform bacteria and 14% contained *E. coli.*

Shrestha (2001) studied the hazard analysis critical control point (HACCP) system in sausage production plants. The result obtained from the microbiological analysis

assessed in eight key stages (raw meat, mixture after filling in casing, after cooking, finished product, spice, casing and water) of sausage production process. After filling in casing (sampleIII) step being the major steps where the highest microbial counts were observed. Pathogenic bacteria such as *Salmonella* spp., *E. coli,* coagulase positive *S. aureus* and *Cl. perfringens* were detected during processing.

The CCP found table showed that the effective Critical Control Points (CCP) was steaming where the microbial hazards can be reduced to completely safe level. Other CCPs are not the effective control points, however, can minimize the microbial loads. The pathogenic microbes were killed during steaming and the steamed *momo* won't get contaminated until and unless served in clean serving plate with spoons and forks.

This study concludes that the hygienic condition of *momo* can be upgraded on the basis of findings of hazards and CCPs. The HACCP approach can be applied to food safety in homes as well as in food processing and food service establishments (WHO/ ICMSF, 1982). HACCP can be applied in food processed in cottage industries to those processed in technically advanced manufacturing plants. It too can be applied in canning industries, meat industries, poultry husbandry, fish industries, dairy industries, fermentation industries which are the sources of producing large amount of various edible products.

6.2 CONCLUSION

Hazard Analysis Critical Control Point (HACCP) was conducted of the fast food *momo* (buff and chicken) from eight different locations of Kathmandu Metropolitan City along with the surveillance study of those restaurants. This study gave the conclusion that the final steamed product both chicken and buff *momo* analysed showed insignificant total aerobic mesophilic count but did not show other microbiological counts such as yeasts and molds count, coliform count and staphylococcal count which may be due to the adequate steaming time and temperature required to kill all the vegetative cells.

During preparation of chicken *momo*, the highest aerobic mesophilic count, total yeasts and molds count, total coliform count and *S. aureus* count were found to be 2.8×10^6cfu/gm, 2.1×10^3cfu/gm, 1.92×10^5cfu/gm and 3.4×10^3cfu/gm respectively. These values and near to these values were obtained from the samples of pickles, spices, raw *momo*, raw meat and mixture of minced meat with spices. Similarly, during preparation of buff *momo*, the highest total aerobic mesophilic count, total yeasts and molds count, coliform count and *S. aureus* counts were found to be 2.82×10^6cfu/gm, 1.9×10^3cfu/gm, 2.1×10^5cfu/gm and 2.8×10^3cfu/gm respectively and these values too were from the samples such as pickles, spices, raw *momo*, mixture of minced meat with spices and raw meat.

From this study it was concluded that the steaming or the cooking of the *momo* is the effective critical control point (CCP) which if carried out for proper time and temperature can eliminate all the possible microbial hazards. The study also focuses that the personal health hygiene of the food handlers and the sound knowledge in sanitation and health hazard issues due to consumption of unhygienic foods should be well understood by these employees too.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

Hazard analysis of fast food (buff *momo* and chicken *momo*) was conducted in eight different fast food restaurants of Kathmandu city. The investigation comprised of observing all steps of preparation, monitoring time temperature throughout processing and collecting samples of raw materials (used as ingredients for preparation of *momo*); different stages in preparation process and the finished final product (steamed *momo*). All these samples were microbiologically investigated to determine the Critical Control Points (CCPs) and whether or not the control measures were implemented properly to alleviate the hygienic condition of these foods. Sanitation is one of the crucial factors that play an important role to detect the hazard analysis of restaurant foods. So, swab of the utensils were taken to detect whether or not the utensils used were properly cleaned or sanitized. Similarly, air environment of the restaurant also determines the hygienic condition of the restaurant which was further investigated by plate exposure method.

The total aerobic mesophilic count, coliform count, staphylococcal count, yeasts and molds count were performed in different stages of *momo* preparation process.

- It was found that after steaming *momo* for 15-20 minutes at 75°C-80°C was sufficient to destroy the pathogenic microorganisms.
- During preparation of chicken *momo*, the highest aerobic mesophilic count, total yeasts and molds count, total coliform count and *S. aureus* count were found to be 2.8×10^6cfu/gm, 2.1×10^3 cfu/gm, 1.92×10^5 cfu/gm and 3.4×10^3 cfu/gm respectively.
- These values and near to these values were obtained from the various samples of pickles, spices, raw momo, raw meat and mixture of minced meat with spices. But the sample of flour (P6) taken showed the minimum value like 5.5×10^4cfu/gm in

total aerobic mesophilic count, 2 cfu/gm in yeasts and molds count, 3×10^1cfu/gm in coliform count and 2×10^1cfu/gm in case of *S. aureus* count.

- While preparation of buff *momo*, the highest total aerobic mesophilic count, total yeasts and molds count, coliform count and *S. aureus* count were found to be 2.82×10^6cfu/gm, 1.9×10^3cfu/gm , 2.1×10^5cfu/gm and 2.8×10^3cfu/gm respectively.
- The obtained values and approximate to these values were obtained from the samples of raw momo, pickles, spices, mixture of minced meat with spices, raw meat. The lowest total aerobic mesophilic count, coliform count, *S. aureus* count and yeasts and molds count were found to be 6×10^4cfu/gm, 4×10^1cfu/gm, 3×10^1cfu/gm and 4cfu/gm from sample (P14) flour.
- *Salmonella* spp. was not isolated from any of the samples taken during preparation of chicken *momo* and buff *momo*.
- *E.*coli was isolated from the samples taken from the restaurant B of Kalanki, restaurant C of Chabahil, restaurant D of Khichapokhari and restaurant G of Balaju during chicken momo preparation. The samples contaminated with *E.*coli were raw meat, mixture of minced meat with spices, raw *momo*, pickles, and spices.
- The results showed the great resemblance between two types of *momo* (chicken and buff *momo*) preparation process.
- In *momo* preparation process, the Critical Control Point (CCP) was steaming; however Control Points (CPs) could be each and every step in the *momo* preparation process.
- The CCPs identified was cooking time-temperature.
- Analysis of drinking water by MPN showed that restaurant of site D was contaminated by fecal coliform, restaurant of site G by coliforms and restaurants of site A, B, C, E, F and H were free of coliform.
- The plate exposure showed that air environment of all the restaurants were similar and the predominant types of microorganisms found were *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Pseudomonas* spp. in descending order.

- The chop boards, the serving plate, the storage vessel for meat and the meat mincer were analyzed for presence of S. *aureus* which gave the positive result.

Besides this, field observation and interview was also conducted in 8 different restaurants with the help of structured questionnaire. The study is summarized as-

- The survey results revealed that the knowledge of sanitation among the restaurant owners and employees were limited.
- Hand washing practices before preparation of foods was observed only in 62% of the restaurant but was not observed during serving of food.
- The microbial safety can be guaranteed when the overall processing, handling and personal hygiene are taken into considerations. Thus, it may help in upgrading the hygienic standard of fast food restaurants of Kathmandu Metropolitan City.

7.2 RECOMMENDATIONS

Based on the above findings, the following recommendations are made:

1. Personal hygiene of the food handler

The food handlers and the food service personnel are an important source of contamination in a food service establishment. Therefore, personal hygiene is the key factor in food safety practices.

2. Storage and preservation

Perishable and non-perishable raw materials should be stored separately and safely.

3. Preparation and cooking

Cooking should be thorough and reach the temperature so that coliform and other harmful pathogens are destroyed.

4. Washing and cleaning practices

The utensils used in the preparation and serving of food should be washed thoroughly with clean water and detergent before and after use as well as the mops used for cleaning should be effectively decontaminated and the hand towels and the dish towels due to of sanitary risks should also be regularly washed and monitored.

5. Cleaning and sanitization of premises

Kitchen and refrigerator shelves are of special importance as sources of contaminants of cooked foods. Similarly, walls, ceilings, window sills, racks, floors subjected to fluid wastes from cooking kettles should be cleaned properly as they too can harbor pathogenic microorganisms.

6. Food safety education and consumer awareness activities

Training and educating the processors and food handlers is one of the most effective interventions to assure the safety of food service establishments. Moreover, food hygiene regulation should be implemented in all the food service establishment and slaughter house regulations, premises; equipment should also be implemented in order to cover the broad aspect of the quality. Consumers should be made aware about the pros and cons of eating outside.

CHAPTER – VIII

8. REFERENCES

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